Effect of dominant fungi on deterioration of protein content in different species of *Cassia* seeds sample

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Abstract:

The present study was aimed to study the seed mycoflora and assess and its effect on protein content of four species of *Cassia* viz, *Cassia fistula* Linn, *Cassia occidentalis* Linn, *Cassia sophera* Linn and *Cassia tora* Linn. Test fungi (*Aspergillus niger* and *Curvularia lunata)* were selected on the basis of their percentage frequency and dominance over the seed surface. Sterilized healthy seeds were artificially infested asceptically with one ml spore suspension and incubated for 15, 30 & 45 days at room temperature Control was maintained by adding one ml sterilized distilled water instead of spore suspension. After incubation seeds were dried at 60°C for 48 hrs then taken for qualitative and quantitative analysis of protein, results shows the protein content decreased gradually after 15, 30 and 45 days of incubation.

In *Cassia fistula* seeds contains 48.12 μg/mg protein content which showed decreasing trend under infestation by *Aspergillus niger* and *Curvularia lunata* with increasing incubation period. In *Cassia occidentalis* was infested with *Aspergillus niger* and *Curvularia lunata* the protein content decreased gradually after 15, 30 and 45 days of incubation respectively. In *Cassia sophera* the protein content was 49.04 μg/mg but gradually decreased under infestation. And in case of *Cassia tora* contained 30.73 μg/mg protein which gradually decreased under infestation.

Key Word: dominant fungi, protein, deterioration and *Cassia species* seeds

Seed deterioration can be defined as deteriorative alterations occurring with time that increase the seed exposure to external challenges and decrease the ability of the seed to survive (Malik and Jyoti, 2013). Manoharachary and Kunwar,(2006); Kapoor et al., (2010) described deterioration as catabolic process which involves cytological, physiological, biochemical and physical changes in seeds. The world s stored grain is damaged mainly by the activity of fungi than other microorganisms was stated by Neergard, (1977). Fungi have assumed great economic significance, as they not only cause spoilage of grains, during pre and post-harvest stages of production, but also produce various toxins (mycotoxins).

There are several reports indicating the significant impact of moisture on the growth and development of moulds and corresponding loss caused by them on the substrate (Prasad and

Bilgrami, 1973; Bilgrami *et al*., 1976, Prasad, 1977; Prasad and Roy 1979; Poddar, 1980; Dwivedi *et al*., 1982; Choubey, 1984, Dutta, 1988). Kumari (1983), Dutta (1988), Kumari *et al*. (1989), Chaourasia (1990), and Ahmad (1992) observed deterioration of proteins, carbohydrates, alkaloids and phenols of some seeds and fruits under infestation at various relative humiditie. Likewise, the influence of temperature on bio-deterioration of active ingredients due to naturally growing fungi as well as individual fungi has well been established, (Bottomley *et al*., 1950; Glass *et al*., 1959; Ghosh *et al*., 1981 and Maheshwari, 1987).The present study was aimed to study the seed mycoflora and assess its effect on protein content of four *Cassia* sp viz, seeds of *Cassia* sp. viz. *Cassia fistula* Linn, *Cassia occidentalis* Linn, *Cassia sophera* Linn and *Cassia tora* Linn.

MATERIALS AND METHODS:

COLLECTION OF SEED SAMPLES:

Collection of seeds of *Cassia* sp. viz. *Cassia fistula*, *Cassia occidentalis*, *Cassia sophera* and *Cassia tora*. were made after maturity from different regions of Bihar representing eastern, western, southern, northern and central zones like Purnia, Muzaffarpur, Gaya, Patna, Lakhisarai, Munger and Bhagalpur directly from the place of occurrences. The seed samples were properly dried and stored separately in different containers viz, glass container, earthen pot, plastic container, steel container and on the open floor under room condition for further investigation.

DETERIORATION OF PROTEIN DUE TO TEST FUNGI UNDER STORAGE

Test fungi were selected on the basis of their percentage frequency and dominance over the seed surface. *Aspergillus niger* and *Curvularia lunata* were selected as test fungi. During infestation, 10 gm of surface sterilized healthy seeds were artificially infested asceptically with spore suspension (1 ml), prepared in sterilized distilled water from one week old culture of the fungus. No. of spores per ml was adjusted by dilution method to approx 200 – 250 spores/ml. The flasks (having seeds) were incubated for 15, 30 & 45 days at room temperature (Ward and Diener, 1961). Control was maintained by adding one ml sterilized distilled water instead of spore suspension. The infested and control seeds were taken up after different incubation periods. They were washed in running water to remove the mycelium and conidia of the fungi. Subsequently they were dried at 60°C for 48 hrs. The dried seeds were then taken for qualitative and quantitative analysis of protein.

BIOCHEMICAL SCREENING

1 gm seed sample of each species was crushed along with 5 ml of 80% ethanol separately in glass mortor pestle. Filtered and used it for protein test.

3 ml of seed extract was taken and added Biuret reagent (3 ml of 20% KOH solution, followed by the addition of 1 ml 5% $CuSO_4$ solution). Development of violet colour indicated the presence of protein.

QUANTITATIVE ESTIMATION OF PROTEIN

The soluble protein content was estimated by F.C. (Folin Ciocalteau) reagent (Lowry *et al*., 1951). 1 ml of seeds homogenate (0.1 gm / ml in Buffer solution) was taken in a centrifuge tube and 3 ml of 10% Trichloroacetic acid (TCA) was added. It was centrifuged for 20 minutes at 3000 rpm. The supernatant was discarded and centrifuge tube were left overnight in an inverted position. 5 ml 0.1 N NaOH was added to centrifuge tube and mixed with the help of cyclomixture. 0.5 ml of the above suspension was taken in a test tube and made the volume to 1 ml by distilled water (DW). Then 5 ml of alkaline copper sulphate reagent (50 parts of 2% Na_2CO_3 in 0.1 N NaOH and 1 part of 0.5% of $CuSO_4.5H_2O$ in 1% sodium potassium tartarate) was added to the above tube and mixed thoroughly. Thereafter, 0.5 ml Folin Ciocalteau (F.C.) reagent was added and mixed. Finally, this mixture was allowed to stand for 30 minutes for optimum colour development and its absorbance was recorded at 600 nm against reagent blank.

The amount of protein was calculated against the standard curve of albumin and expressed as µg / mg seed on fresh weight basis. Data recorded at each stage are the mean of six different replicates for each sample. ANOVA test was made among various parameters at different stage of storage conditions and infested seed samples.

RESULT AND DICUSSION

Microorganisms also depend solely for their growth and development on proteins which serve as the major source of nitrogen for them. Wacksman and Starkey (1932) and Jermyn (1953) reported that gelatin; casein and albumin provide the source of nitrogen for the moulds, while dipeptides and tripeptides as source of nitrogen for *Aspergillus* sp. have been reported by Cochrane (1958) and Bilgrami *et al*., (1979a, 1979b and 1979c). Cherry *et al*. (1974) observed the decomposition of high molecular weight protein globulins like conarchin and arachin in *Arachis hypogea* to smaller components by *Aspergillus parasiticus*, leading to reduction in the quantity of protein contents. Utilization of proteins by moulds depends on their efficacy to convert it into amino acids. Changes in protein contents of the host under pathogenesis have also been investigated by Jhonson *et al*. (1966, 1968) and Prasad *et al*. (1976) in leaves. In the present study, therefore, attempts have been made to find out the changes in the protein contents due to fungal infestations.

A gradual decrease in protein contents were observed due to both the test fungi viz. *Aspergillus niger and Curvularia lunata* (Detailed result showed in Table).

Cassia fistula seeds sample contains 48.12 µg/mg protein content which showed decreasing trend under infestation by *Aspergillus niger* and *Curvularia lunata* with increasing incubation period. In *Cassia fistula* seeds after infestation with *Aspergillus niger* the protein content decreased gradually to 46.98, 39.44 and 35.91 µg/mg after 15, 30 and 45 days of incubation and due to infestation with *Curvularia lunata* it reduced to 45.97, 36.70 and 36.35 µg/mg after 15, 30 and 45 days of incubation. Minimum deterioration of protein contents was 0.085% after 15 days of incubation whereas maximum deterioration was 8.32% after 45 days of incubation when infested by *Aspergillus niger*. When seeds were infestated by *Curvularia lunata* minimum deterioration was 2.23% after 15 days and maximum deterioration was 8.18% after 30 days of maintained 43.37 µg/mg protein content which gradually decreased under infestation incubation period.

When seeds of *Cassia occidentalis* was infested with *Aspergillus niger* and *Curvularia lunata* the protein content decreased gradually to 33.36, 31.50 and 29.50 µg/mg and to 30.20, 28.90 and 26.60 µg/mg after 15, 30 and 45 days of incubation respectively. Minimum deterioration of protein contents was 19.64% over control after 30 days of incubation and maximum deterioration was 24.35% over control after 45 days of incubation period due to infestation by *Aspergillus niger*. *Curvularia lunata* infested seeds showed minimum deterioration to the extent of 26.27% over control after 30 days of incubation and maximum to 31.79% over control after 45 days of incubation period.

In dried seeds of *Cassia sophera* the protein content was 49.04 µg/mg but gradually decreased under infestation. Due to infestation by *Aspergillus niger* in *Cassia sophera* seeds the protein content decreased gradually to 37.48, 37.39 and 35.96 µg/mg and due to *Curvularia lunata* infestation it decreased to 39.99, 39.83 and 38.91 µg/mg after 15, 30 and 45 days of incubation. Minimum deterioration of protein contents (12.14% change over control) was after 30 days of incubation and maximum (22.65% over control) was after 15 days of incubation period when seeds were infested with *Aspergillus niger*. Due to infestation by *Curvularia lunata* minimum deterioration (5.44% over control) was after 45 days of incubation and maximum (17.47% over control) was after 15 days of incubation.

The dried seeds of *Cassia tora* contained 30.73 µg/mg protein which gradually decreased under infestation. *Aspergillus niger* infested seeds of *Cassia tora* showed decrease in the protein content. It decreased gradually to 26.60, 20.10 and 16.23 µg/mg when infested with *Aspergillus niger* and due to infestation by *Curvularia lunata* it decreased to 24.99, 24.00 and 23.36 µg/mg after 15, 30 and 45 days of incubation period. After infestation with *Aspergillus niger* the minimum deterioration of protein contents was 13.18% (over control) after 15 days of incubation and maximum deterioration was 38.02% (over control) after 45 days of incubation period. Minimum deterioration 10.80% (over control) was observed after 45 days of incubation and

maximum 20.13% (over control) after 30 days of incubation period when seeds of *Cassia tora* was infested with *Curvularia lunata*.

Analysis of variance Table – 26 shows that the effect of incubation period and infestation of fungi in *Cassia fistula*, *Cassia occidentalis*, *Cassia sophera* and *Cassia tora* seeds were significant at 1% level of significance.

Table: Change in Protein (µg/mg) contents of *Cassia fistula* seed sample due to infestation by fungi

Incubation date	*Cassia fistula*			*Cassia occidentalis*			*Cassia sophera*			*Cassia tora*		
	C	An	Cu	C	An	Cu	C	An	Cu	C	An	Cu
0 Day	48.12	48.12	48.12	43.37	43.37	43.37	49.04	49.04	49.04	30.73	30.73	30.73
15 Day	47.02	46.98	45.97	42.48	33.36	30.2	48.46	37.48	39.99	30.64	26.6	24.99
% change over control	2.28	0.085	2.23	2.05	21.46	28.9	1.18	22.65	17.47	0.29	13.18	18.43
30 Day	39.97	39.97	36.7	39.2	31.5	28.9	42.56	37.39	39.83	30.05	20.1	24
% change over control	16.93	1.32	8,18	9.61	19.64	26.27	13.21	12.14	6.41	2.21	33.11	20.13
45 Day	39.17	35.91	36.35	39	29.5	26.6	41.15	35.96	38,91	26.19	16.23	23.36
% change over control	18.59	8.32	7.19	30.07	24.35	31.79	16.08	12.61	5.44	14.77	38.02	10.8

The results show that, all common and dominant seed-borne fungi of cassia seeds caused reduction in protein content. Gradual deterioration in the protein level in all the infested test samples supports the findings of Srivastavaand Sweta (2013) reported maximum protein content of Jatropha crucas L. was reduced by Seed-borne fungi *Aspergillus flavus*, *A. niger*. A gradual loss of carbohydrate (both soluble and insoluble) and protein was recorded due to storage fungi of maize, groundnut and soybea. Singh *et al*. (1973); Sharma and Wahab (1975); Jamaluddin *et al*. (1977); Bhargava and Sukla (1980); Singh and Sinha (1982); Ojha (1983); Prasad and Das (1985); etc. Similarly, Reisener and Zieglar (1970); Reisener *et al*. (1972) and Cherry *et al*. (1975) reported that amino acids produced in the host by the break-down of proteins are being utilized by fungi for the synthesis of their own protein. Chemical and physiological changes in various kinds of proteins under infestation have been observed due to enzymatic activity of the pathogen (Uritani, 1971).

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